Claims

We claim:

1. A polynucleotide comprising a polynucleotide sequence encoding a mutant small subunit of a heat labile plant ADP glucose pyrophosphorylase (AGP) enzyme, or a fragment thereof, wherein said small subunit comprises a mutation in the N-terminal portion thereof, and wherein when said mutant small subunit is expressed with a large subunit of a plant AGP enzyme to form a mutant enzyme, said mutant enzyme exhibits increased heat stability relative to a wild type AGP enzyme.

2. The polynucleotide according to claim 1, wherein said mutant small subunit is a maize AGP subunit.

3. The polynucleotide according to claim 2, wherein said mutant small subunit is a maize endosperm AGP small subunit comprising an amino acid mutation wherein the tyrosine amino acid at position 36 of the wild type maize endosperm AGP small subunit sequence is replaced with an amino acid that confers said increased heat stability on said mutant enzyme.

4. The polynucleotide according to claim 3, wherein said tyrosine is replaced by a cysteine.

5. The polynucleotide according to claim 4, wherein said mutant small subunit comprises the amino acid sequence shown in SEQ ID NO:4.

6. The polynucleotide according to claim 5, wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:3.

7. The polynucleotide according to claim 3, wherein said mutant small subunit comprises a further mutation wherein an amino acid is inserted between the serine amino acid at position 34 and the threonine amino acid at position 35 of the wild type maize endosperm AGP small subunit sequence.

8. The polynucleotide according to claim 7, wherein said tyrosine is replaced by a cysteine.

9. The polynucleotide according to claim 7, wherein the inserted amino acid is a glutamine.

10. The polynucleotide to claim 8, wherein the inserted amino acid is a glutamine.

11. The polynucleotide according to claim 10, wherein said mutant small subunit comprises the amino acid sequence shown in SEQ ID NO:8.

12. The polynucleotide according to claim 11, wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:7.

13. The polynucleotide according to claim 7, wherein the inserted amino acid is a glutamic acid.

14. The polynucleotide according to claim 8, wherein the inserted amino acid is a glutamic acid.

15. The polynucleotide according to claim 14, wherein said mutant small subunit comprises the amino acid sequence shown in SEQ ID NO:10.

16. The polynucleotide according to claim 15, wherein said polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:9.

17. The polynucleotide according to any preceding claim, wherein said polynucleotide comprises a polynucleotide sequence encoding a large subunit of a plant AGP enzyme.

18. The polynucleotide according to claim 17, wherein said large subunit comprises a mutation that confers increased heat stability on an AGP enzyme that comprises said large subunit.

19. The polynucleotide according to claim 18, wherein said large subunit comprises a heat stability (*HS*) mutation selected from the group consisting of *HS13*, *HS14*, *HS16*, *HS33*, *HS40*, *HS47*, *HS RTS 48-2*, *HS RTS 60-1*, *HS33F*, *HS33M*, *HS7+3*, *HS6+3*, *HS7+6*, and *HS7+6+3*.

20. A method for increasing resistance of a plant to heat stress conditions, said method comprising incorporating the polynucleotide of any preceding claim into the genome of a plant and expressing the protein encoded by said polynucleotide, thereby increasing resistance of the plant to heat stress conditions.

21. The method according to claim 20, wherein said plant is a monocotyledonous plant.

22. The method according to claim 21, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

23. The method according to claim 20, wherein said plant is *Zea mays*.

24. The method according to claim 20, wherein said plant is a dicotyledonous plant.

25. The method according to claim 24, wherein said dicotyledonous plant is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

26. The method according to claim 20, wherein said plant comprises or expresses a large subunit of a plant AGP enzyme, wherein said large subunit comprises an amino acid mutation that confers increased seed weight to a plant comprising or expressing said large subunit.

27. The method according to claim 26, wherein said large subunit comprises the *Rev6* mutation.

28. The method according to claim 26, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large AGP subunit of maize.

29. The method according to claim 26, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

30. The method according to claim 26, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type AGP large subunit of maize.

31. A plant or plant tissue comprising the polynucleotide molecule of any of claims 1-19.

32. The plant or plant tissue according to claim 31, wherein said plant or plant tissue is monocotyledonous.

33. The plant or plant tissue according to claim 32, wherein said monocotyledonous plant or plant tissue is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

34. The plant or plant tissue according to claim 31, wherein said plant is *Zea mays* or said plant tissue is from *Zea mays*.

35. The plant or plant tissue according to claim 31, wherein said plant or plant tissue is dicotyledonous.

36. The plant or plant tissue according to claim 35, wherein said dicotyledonous plant or plant tissue is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover,

kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

37. The plant or plant tissue according to claim 31, wherein said plant tissue is a seed.

38. The plant or plant tissue according to claim 31, wherein said plant comprises or expresses a large subunit of a plant AGP enzyme, wherein said large subunit comprises an amino acid mutation that confers increased seed weight to a plant comprising or expressing said large subunit.

39. The plant or plant tissue according to claim 38, wherein said large subunit comprises the *Rev6* mutation.

40. The plant or plant tissue according to claim 38, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

41. The plant or plant tissue according to claim 38, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

42. The plant or plant tissue according to claim 38, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type AGP large subunit of maize.

43. A composition comprising:

i) a polynucleotide as defined in any of claims 1-16; and

ii) a polynucleotide comprising a polynucleotide sequence that encodes a large subunit of a plant AGP enzyme.

44. The composition according to claim 43, wherein said large subunit comprises a mutation that confers increased heat stability on an AGP enzyme that comprises said large subunit.

45. The composition according to claim 44, wherein said large subunit comprises a heat stability (HS) mutation selected from the group consisting of HS13, HS14, HS16, HS33, HS40, HS47, HS RTS 48-2, HS RTS 60-1, HS33F, HS33M, HS7+3, HS6+3, HS7+6, and HS7+6+3.

46. A polypeptide encoded by a polynucleotide as defined in any of claims 1-16.

47. An AGP enzyme comprising a polypeptide as defined in claim 46.

48. The AGP enzyme according to claim 47, comprising a large subunit of a plant AGP enzyme, wherein said large subunit comprises a mutation that confers increased heat stability on an AGP enzyme that comprises said large subunit.

49. The AGP enzyme according to claim 48, wherein said large subunit comprises a heat stability (HS) mutation selected from the group consisting of HS13, HS14, HS16, HS33, HS40, HS47, HS RTS 48-2, HS RTS 60-1, HS33F, HS33M, HS7+3, HS6+3, HS7+6, and HS7+6+3.

50. The AGP enzyme according to claim 47, wherein said plant comprises or expresses a large subunit of a plant AGP enzyme, wherein said large subunit comprises an amino acid mutation that confers increased seed weight to a plant comprising or expressing said large subunit.

51. The AGP enzyme according to claim 50, wherein said large subunit comprises the *Rev6* mutation.

52. The AGP enzyme according to claim 50, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

53. The AGP enzyme according to claim 50, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

54. The AGP enzyme according to claim 50, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type AGP large subunit of maize.

55. The AGP enzyme according to claim 48, wherein said plant comprises or expresses a large subunit of a plant AGP enzyme, wherein said large subunit comprises an amino acid mutation that confers increased seed weight to a plant comprising or expressing said large subunit.

56. The AGP enzyme according to claim 55, wherein said large subunit comprises the *Rev6* mutation.

57. The AGP enzyme according to claim 55, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

58. The AGP enzyme according to claim 55, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

59. The AGP enzyme according to claim 55, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type AGP large subunit of maize.

60. A method for preparing a plant having an AGP enzyme that exhibits increased stability relative to a wild type AGP enzyme said method comprising introducing a polynucleotide as defined in any of claims 1-19 into a plant cell and growing a plant from said plant cell.

61. The method according to claim 60, wherein said plant grown from said plant cell is selected for expression of said polynucleotide.

62. The method according to claim 60, wherein said plant is a monocotyledonous plant.

63. The method according to claim 22, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

64. The method according to claim 60, wherein said plant is *Zea mays*.

65. The method according to claim 60, wherein said plant is a dicotyledonous plant.

66. The method according to claim 65, wherein said dicotyledonous plant is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

67. The method according to claim 60, wherein said plant comprises or expresses a large subunit of a plant AGP enzyme, wherein said large subunit comprises an amino acid mutation that confers increased seed weight to a plant comprising or expressing said large subunit.

68. The method according to claim 67, wherein said large subunit comprises the *Rev6* mutation.

69. The method according to claim 67, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large AGP subunit of maize.

70. The method according to claim 67, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type AGP large subunit of maize.

71. The method according to claim 67, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type AGP large subunit of maize.

72. An expression construct comprising a polynucleotide as defined in any of claims 1-19.

73. The expression construct according to claim 72, wherein said expression construct comprises a regulatory element operably linked to said polynucleotide.

74. The expression construct according to claim 73, wherein said regulatory element is selected from the group consisting of a promoter, transcription termination sequence, translation termination sequence, enhancer, and polyadenylation sequence.

75. The expression construct according to claim 74, wherein said promoter is a promoter functional in a plant cell.

76. The expression construct according to claim 75, wherein said promoter is a seed-specific promoter, a tissue-specific promoter, a constitutive promoter, a developmentally-regulated promoter, or an inducible promoter.

77. The expression construct according to claim 76, wherein said seed-specific promoter is a β-phaseolin gene promoter or a glycinin gene promoter.

78. The expression construct according to claim 76, wherein said constitutive promoter is a CaMV promoter, ubiquitin promoter, actin promoter, or NOS promoter.

79. The expression construct according to claim 76, wherein said tissue-specific promoter is an E8 promoter.

80. The expression construct according to claim 75, wherein said promoter is selected from the group consisting of a CaMV 35S promoter, CaMV 19S promoter, prolifera promoter, Ap3 promoter, heat shock promoter, T-DNA 1'- or 2'-promoter of *A. tumafaciens*, polygalacturonase promoter, petunia chalcone synthase A (CHS-A) promoter, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter, maize WipI promoter, maize trpA gene promoter, maize CDPK gene promoter, and RUBISCO SSU promoter.

81. The expression construct according to claim 72, wherein said expression construct comprises a selectable marker gene.

82. The expression construct according to claim 81, wherein said gene is selected from the group consisting of a gene encoding antibiotic resistance and a gene encoding herbicide resistance.

83. The expression construct according to claim 82, wherein said antibiotic resistance gene is selected from the group consisting of hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, neomycin, and spectinomycin.

84. The expression construct according to claim 82, wherein said herbicide resistance gene is a gene that provides resistance to phosphinothricin acetyltransferase or glyphosate.

85. The expression construct according to claim 81, wherein said gene is selected from the group consisting of genes encoding β-glucuronidase (GUS), β-galactosidase, luciferase, nopaline synthase, chloramphenicol acetyltransferase (CAT), green fluorescence protein (GFP), and enhanced GFP.